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DECLARATION UNDER 37 CFR 1.132

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WASHINGTON, D.C. 20231

SIR:

Now comes Yoshiharu MATAHIRA who deposes and says that:

1. I am a co-inventor of the invention described and claimed in the above-referenced application.
2. I graduated from Shizuoka University, Faculty of Agriculture, Department of Agricultural Chemistry in 1984, and received my doctoral degree in agriculture from Gifu University. United Graduate School, Agricultural Research Course in 1995, and has been employed by Yaizu Suisan Kagaku Industry Co., Ltd. since 1984.
3. Under my supervision and control, the following experiments were carried out:

### TEST EXAMPLE

The following tests were carried out to confirm the effects of anserine and D-ribose used in combination, using the under-mentioned reagents:

Anserine hydrochloride (prepared by Yaizu Suisan Kagaku Industry Co., Ltd. in accordance with the method as described in "Preparation Example 1 (Preparation of anserine) on page 8, line 22 to page 9, line 17 of the present specification)

D-glucose (Wako Junyaku Co., Ltd.)

D-fructose (Wako Junyaku Co., Ltd.)

D-ribose (Wako Junyaku Co., Ltd.)

80 SPF mice (male) of 6-weeks old were separated into eight groups (one control group and seven test groups; each group consists of 10 mice), and after 4 hours fasting, oral administration was forcedly made so that water for injection would be applied to the control group (Group 1) in an amount of 200 mg/kg of body weight, and aqueous solutions of the above reagents would be applied to the test groups as indicated below:

Anserine-administration group (Group 2): An aqueous solution of anserine hydrochloride (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of anserine hydrochloride

Glucose-administration group (Group 3): An aqueous solution of D-glucose (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of D-glucose

Fructose-administration group (Group 4): An aqueous

solution of D-fructose (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of D-fructose

Ribose-administration group (Group 5): An aqueous solution of D-ribose (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of D-ribose

Anserine/glucose mixture-administration group (Group 6): An aqueous solution of a mixture of anserine hydrochloride and D-glucose at a mass ratio of 1/1 (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of the total amount of anserine hydrochloride and D-glucose

Anserine/fructose mixture-administration group (Group 7): An aqueous solution of a mixture of anserine hydrochloride and D-fructose at a mass ratio of 1/1 (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of the total amount of anserine hydrochloride and D-fructose

Anserine/ribose mixture-administration group (Group 8): An aqueous solution of a mixture of anserine hydrochloride and D-ribose at a mass ratio of 1/1 (40 mg/ml) applied in an amount of 200 mg/kg of body weight in terms of the total amount of anserine hydrochloride and D-ribose

Accurately 1 hour after the oral administration, the mice were loaded with the following forced exercise. Mice were put into a water bath (W 265mm x D 427mm x H 204mm) containing water of 20°C, wherein the water surface was made choppy by blowing air, and the swimming time was measured. Each mouse was loaded with a weight which corresponds to 10% of the average body weight

of mice, and the swimming time was represented by the time from the start of swimming until the head of the mouse submerged for at least 7 seconds. The results are indicated in the graph of Fig.1.

Further, when 1 hour passed after the loading of exercise, the blood was collected and the plasma was separated, and the lactic acid amount in the plasma was measured. The measurement of lactic acid amount was carried out with a commercially available kit (trade name: "F-kit L-lactic acid"; manufactured by Beringer Mannheim Co.). Results of respective measurements were represented by an average value  $\pm$  standard deviation ( $n = 10$ ), and examination of significance was carried out by Student's t-test. The results are indicated in the graph of Fig.2.

From the results as indicated in the graph of Fig.1, it is found that the anserine/ribose mixture-administration group (Group 8) shows a longer swimming time than the control group and other test groups.

Fig.2 shows the lactic acid amount in the plasma when one hour passed from the completion of the loading of exercise. From the results of the graph of Fig.2, it is found that the anserine/ribose mixture-administration group (Group 8) shows the lowest lactic acid amount in the plasma as compared with the control group and other test groups.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: \_\_\_\_\_

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Yoshiharu MATAHIRA